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First report of *Colletotrichum fioriniae* infection of hazelnut

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Abstract—In a 2008–09 study of hazelnut fruit cluster diseases in Ordu, Giresun, and Trabzon provinces of Turkey, brown to black sunken, circular to irregular lesions and rots were observed, usually at the bottom end of the hazelnut cluster, close to the pedicel. *Colletotrichum* species were most frequently isolated from the lesions, and 13 isolates were identified morphologically as *C. fioriniae*, and the identity of one of them was confirmed by molecular analyses. Pathogenicity tests were conducted under laboratory, greenhouse, and field conditions, and symptoms matching the original field symptoms were observed on leaves, fruit clusters, and pedicels. This is the first report of *C. fioriniae* as a pathogen of hazelnut (*Corylus avellana*) and the first record of *C. fioriniae* from Turkey.

Key words—Black Sea region, *Colletotrichum acutatum*, *Glomerellaceae*

Introduction

Hazelnut (*Corylus avellana* L.) is one of the most important tree nut crops in Turkey, which with 80% of world production is the leading exporter of hazelnuts (FAOSTAT 2011). Hazelnut production in Turkey in the Black Sea region extends from the Georgia border in the east to Istanbul in the west. Surveys were carried out in 2008–09, on 221 hazelnut orchards located at Ordu, Giresun, and Trabzon provinces in the Black Sea region of Turkey (Fig. 1). Almost 50% of the surveyed orchards had symptomatic clusters with brown to black sunken, circular to irregular lesions that can progress from husk into fruit

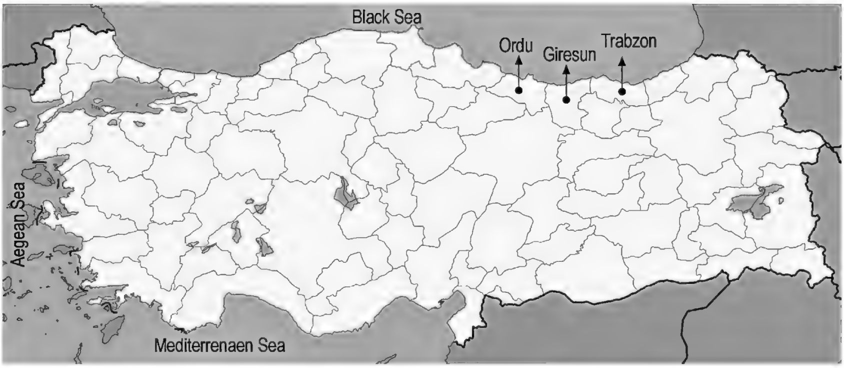


Fig. 1: Provinces surveyed for fruit cluster disease of hazelnut in Turkey.

(Figs 2–4). *Colletotrichum* was the predominant genus isolated (Sezer & Dolar 2012), and 13 morphologically similar isolates were provisionally determined as *C. fioriniae.*

Colletotrichum fioriniae has not previously been reported as a pathogen of hazelnut or from Turkey.

Materials & methods

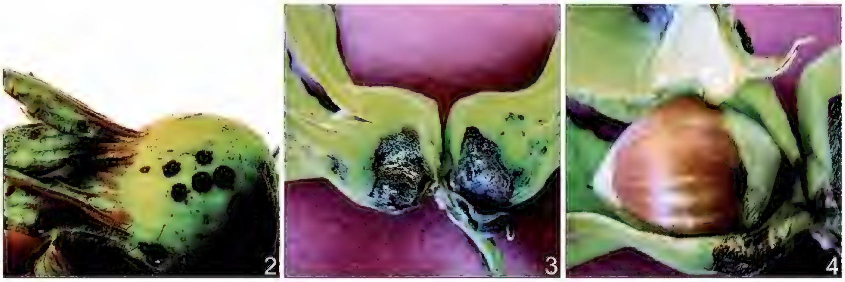
Fungal isolates

During 2008–09, fungi were isolated from symptomatic clusters from different cultivars and locations (Sezer & Dolar 2012). Infected fruit clusters were surface-sterilized (0.5–1% w/v sodium hypochlorite) for 1–2 min before being placed onto potato dextrose agar (PDA, Merck) containing streptomycin. Single spore isolates were obtained from fungal cultures incubated at 23 ± 1°C with a 12 h photoperiod for 3–5 days and stored on PDA slants in tubes at 4°C. Cultures were conserved in the Department of Plant Protection, Ankara University, Ankara, Turkey (AUPP) and the Hazelnut Research Institute, Giresun, Turkey (HRI).

Identification of fungus

Culture morphology, growth rate, and conidial morphology were observed from 7–10-day old cultures grown on PDA (Sutton 1980, Marcelino et al. 2008, Cai et al. 2009, Hyde et al. 2009a,b, Shivas & Tan 2009). Mycelial appressorial characters were observed from slide cultures on Potato Carrot Agar (PCA) (Sutton 1980). Shape, length, and width of 100 conidia were measured and mean length and width were calculated. Initial morphological fungal identification was confirmed by CABI-UK, who sequenced the internal transcribed spacer (ITS) of the rRNA gene of isolate P39.

Additional analyses were made of partial sequences of the beta-tubulin-2 (β-tub), glyceraldehyde 3-phosphate dehydrogenase (GAPDH), and chitin synthase-1 (CHS-1)



Figs 2–4: *Colletotrichum fioriniae*: field symptoms. 2. Small lesions on husk. 3. Large lesions on bottom end of the fruit cluster. 4. Brownish black lesion on fruit shell under the symptomatic husk.

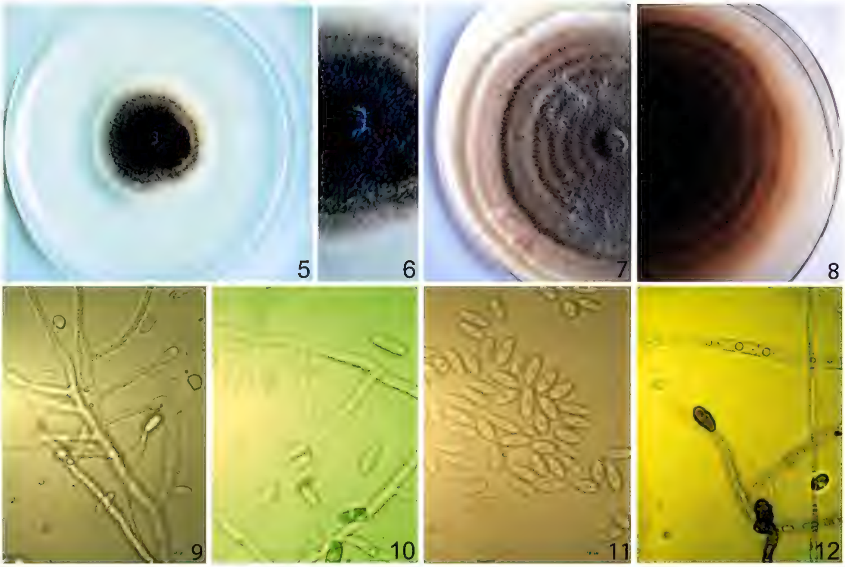
genes (Damm et al. 2009, 2012a,b, 2014; Weir et al. 2012), using universal primers T1/βt2b for β-tub, GDF1/GDR1 for GAPDH, and CHS1-79F/CHS1-354R for CHS-1 (Glass & Donaldson 1995, Carbone & Kohn 1999, Guerber et al. 2003). For DNA extraction approximately 300 mg mycelium were harvested and ground with liquid nitrogen in a sterile mortar from culture medium. Genomic DNA was extracted using a Qiagen DNeasy ® Plant Mini Kit, as specified by the manufacturer, and stored at –20°C prior to use. PCR reaction mixtures and condition were modified from previous studies (Aroca & Raposo 2007, Cobos & Martin 2008). The reaction mixtures of PCR, a final volume of 50 µl, contained 5µl of 10X buffer [75 mM Tris HCl, pH 9.0, 50 mM KCl, 20 mM $(NH_4)2SO_4$], 2 µl of 5 µM each primers, 5 µl of 1.5mM $MgCl_2$, 2 µl of 10 mM deoxynucleoside triphosphates (dNTPs), 1 U Taq polymerase (Fermatas), 5 µl of DNA template for each reaction and 5 µl of bovine serum albumin (BSA: 10 mg/ml). DNA amplifications were carried out in a Techne TC-5000 thermal cycler by the following program: 94°C for 2 min, followed by 34 cycles of [denaturation (94°C for 30 s), annealing (60°C for 30 s), and (3) extension (72°C for 30 s)], and a final extension step 10 min at 72°C. Sequencing was conducted by Genoks (Ankara, Turkey). The nucleotide sequences were subjected to BLAST analysis (http://www.ncbi.nlm.nih.gov) and compared to other sequences in GenBank using CLC Main Workbench 7.0.1 (CLC Bio, Denmark). Our new sequences were deposited in GenBank.

Pathogenicity tests

Pathogenicity tests were conducted on Tombul and Kalınkara hazelnut cultivars using detached leaves in the laboratory, detached twigs and clusters in the greenhouse, and attached twigs in the orchard according to Sezer & Dolar (2012).

Disease assessment

For disease assessment, percentage surface area covered by lesions on leaf or fruit cluster of hazelnut was determined. Disease severity was calculated by estimating lesion sizes on a scale of 0 to 3, where 0 = no lesions, 1 = 1–30% of tissues affected, 2 = 31–60% of tissues affected, and 3 = >60% of tissues affected. These scale values were converted to



Figs 5–12: *Colletotrichum fioriniae*. 5. 5-day old colony on PDA. 6. Enlarged view of spore masses on a 5-day old colony. 7. 10-day old colony on PDA. 8. Reverse side of 10-day old colony. 9. Unicellular conidiophores. 10. Septate conidiophores. 11. Conidia. 12. Appressoria. Scale bars = 10 µm.

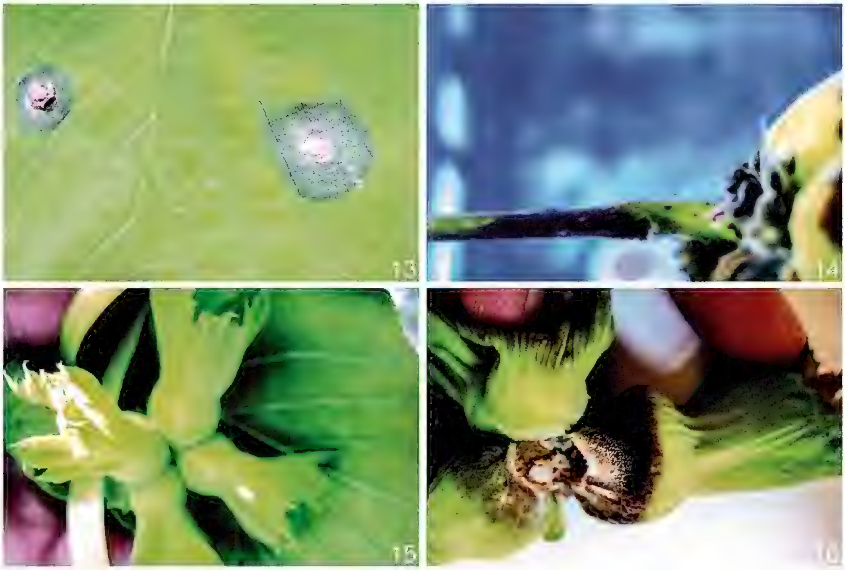
disease severity values (Xi et al. 1990) based on the formula: Disease Severity % = 100 × Σ(no. of plants in category × category number) / (total no. of plants × max. category number).

Results & discussion

With a 30.41% frequency among 217 fungal isolates, *Colletotrichum* was the main genus isolated from symptomatic fruit clusters. Of the thirteen *Colletotrichum* isolates evaluated showing similar cultural and morphological characteristics, one (P39) was also confirmed as *C. fioriniae* by phylogenetic analyses.

Colletotrichum fioriniae (Marcelino & Gouli) Pennycook,
Mycotaxon 132(1): 150. 2017. Fig. 5–12
≡ *Glomerella acutata* var. *fioriniae* Marcelino & Gouli, Mycologia 100: 362. 2008.

Single spore isolates developed colonies on PDA 48.6 mm diam. after 7 days and 71.3 mm diam. after 10 days; the 7–10-day old colonies were circular, slightly raised, dense, and cottony with aerial mycelium in compact tufts



Figs 13–16. *Colletotrichum fioriniae*: hazelnut pathogenicity test symptoms. 13. On detached leaf. 14. On pedicel of the fruit cluster on detached twig. 15. Appearance of fruit cluster 20 days after inoculation (symptomless) in orchard. 16. Symptom on fruit cluster at harvest time in orchard.

and no distinct acervuli. Rarely observed conidial masses were salmon pink. Surface colony color was greenish gray-pink, reverse was pale brown to pink. While the first single spore isolate cultures on PDA were distinctly pink, the color became less intense with subsequent subculturing. Sclerotia and setae were absent. Mycelia were branched, septate, hyaline. Conidiophores formed directly on hyphae, 22.9 ± 1.7 × 2.6 ± 0.1 µm (mean), hyaline, cylindrical, usually unicellular and occasionally branched. Conidia in masses were 11.4 ± 0.2 × 4.1 ± 0.1 µm, hyaline, guttulate, aseptate, straight, usually ellipsoid to fusiform and acute at both ends. Mycelial appressoria (rare in slide cultures) measuring 8.3 ± 0.2 × 5.4 ± 0.1 µm, pale to dark brown, clavate or slightly irregular.

> Specimens examined: **TURKEY, Ordu Province, Perşembe district**, Mersin village, from rotting *Corylus avellana* fruit cluster, July 2008, Arzu Sezer P39 (AUPP, HRI; culture, IMI 500303; GenBank KU736863, KU736864, KU736865).

These morphological characteristics matched the description of *Colletotrichum fioriniae* (Marcelino et al. 2008, as *C. acutatum* var. *fioriniae;* Shivas & Tan 2009), and the rRNA gene ITS sequence from *C. fioriniae* isolate P39 was confirmed as *C. fioriniae* by CABI-UK (IMI 500303). Damm et al. (2012a) recommended that both β-tub and GAPDH genes were suitable for identification of species belonging to the *C. acutatum* complex. BLAST of the DNA sequences obtained from three different gene regions of the isolate P39 showed a high identity (>99%) with previously deposited authentic sequences of *C. fioriniae* in GenBank: β-tub—JN121279, AB690809, GU183268, GU183272, KJ955322, KJ650030; GAPDH—KM252213, KM252196, KF944354, KJ954876; CHS-1—JQ948954, KT153547.

Pathogenicity tests showed that *C. fioriniae* produced large circular lesions on leaf surface. On detached twigs and clusters it caused elongate lesions on the pedicel producing salmon-orange spore masses. At the orchard, no symptoms were observed in inoculated fruit clusters near harvest time. At harvest three months after inoculation, large brown necrotic areas with salmon-orange spore masses on bottom ends of fruit clusters were observed (Figs 13–16). The fungus was re-isolated from all diseased tissues. No symptoms were observed on the controls. Disease severity on detached leaves was 33.33% on Tombul cultivar and 50% on Kalinkara cultivar, 77.78% on detached twigs and clusters, and 41.67% on fruit clusters in the orchard inoculations.

Marcelino et al. (2008) originally described *Colletotrichum fioriniae* as a variety of *C. acutatum* (with a sexual morph, *Glomerella acutata* var. *fioriniae*), and Shivas & Tan (2009) proposed that the *Colletotrichum* and *Glomerella* names should both be elevated to species rank. Unfortunately, only one of these four names was validly published; subsequently, *C. fioriniae* has been validly published, based on the valid basionym *G. acutata* var. *fioriniae* (Pennycook 2017). According to Shivas & Tan (2009) *C. fioriniae* is morphologically similar to *Colletotrichum acutatum* J.H. Simmonds, which differs by having colonies on PDA with a grey cottony aerial mycelium (pale brownish pink with dark flecking in reverse); sparse conidiomata with masses of orange conidia; and narrowly elliptical conidia, 9–15 × 3–4.5 µm.

Previously, *Colletotrichum acutatum* was identified as a pathogen on hazelnut fruit clusters with same symptoms described here in Turkey (Sezer & Dolar 2012); *C. gloeosporioides* (Penz.) Penz. & Sacc. was identified as a pathogen on leaves and stems of hazelnut in Iran (Mirhosseini-Moghaddam & Taherzadeh 2007); and *Colletotrichum* sp. (among other fungi) was isolated from hazelnut flowers/fruits affected by a disease named gray necrosis in Italy (Belisario & Santori 2009). To our knowledge, this is the first report of *C. fioriniae* as a pathogen of *Corylus avellana*. This is also the first report of *C. fioriniae* infection of a crop plant in Turkey.

Acknowledgements

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